

1,5-DISUBSTITUTED IMIDAZOLIDIN-2-ONE DERIVATIVES FOR USE AS EP4 RECEPTOR  
AGONISTS IN THE TREATMENT OF EYE AND BONE DISEASES

This case claims the benefit of provisional application USSN  
60/386,641, filed June 6, 2002.

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## BACKGROUND OF THE INVENTION

Glaucoma is a degenerative disease of the eye wherein the  
intraocular pressure is too high to permit normal eye function. As a result,  
damage may occur to the optic nerve head and result in irreversible loss of  
visual function. If untreated, glaucoma may eventually lead to blindness.  
Ocular hypertension, i.e., the condition of elevated intraocular pressure without  
optic nerve head damage or characteristic glaucomatous visual field defects, is  
now believed by the majority of ophthalmologists to represent merely the  
earliest phase in the onset of glaucoma.

Many of the drugs formerly used to treat glaucoma proved  
unsatisfactory. Early methods of treating glaucoma employed pilocarpine and  
produced undesirable local effects that made this drug, though valuable,  
unsatisfactory as a first line drug. More recently, clinicians have noted that  
many  $\beta$ -adrenergic antagonists are effective in reducing intraocular pressure.  
While many of these agents are effective for this purpose, there exist some  
patients with whom this treatment is not effective or not sufficiently effective.  
Many of these agents also have other characteristics, e.g., membrane stabilizing  
activity, that become more apparent with increased doses and render them  
unacceptable for chronic ocular use and can also cause cardiovascular effects.

Agents referred to as carbonic anhydrase inhibitors decrease the  
formation of aqueous humor by inhibiting the enzyme carbonic anhydrase.  
While such carbonic anhydrase inhibitors are now used to treat elevated  
intraocular pressure by systemic and topical routes, current therapies using  
these agents, particularly those using systemic routes are still not without  
undesirable effects. Topically effective carbonic anhydrase inhibitors are  
disclosed in U.S. Patent Nos. 4,386,098; 4,416,890; 4,426,388; 4,668,697;  
4,863,922; 4,797,413; 5,378,703, 5,240,923 and 5,153,192.

Prostaglandins and prostaglandin derivatives are also known to  
lower intraocular pressure. There are several prostaglandin types, including the  
A, B, C, D, E, F, G, I and J- Series (EP 0561073 A1). U.S. Patent 4,883,819 to

Bito describes the use and synthesis of PGAs, PGBs and PGCs in reducing intraocular pressure. U.S. Patent 4,824,857 to Goh et al. describes the use and synthesis of PGD<sub>2</sub> and derivatives thereof in lowering intraocular pressure including derivatives wherein C-10 is replaced with nitrogen. U.S. Patent 5,001,153 to Ueno et al. describes the use and synthesis of 13,14-dihydro-15-keto prostaglandins and prostaglandin derivatives to lower intraocular pressure. U.S. Patent 4,599,353 describes the use of eicosanoids and eicosanoid derivatives including prostaglandins and prostaglandin inhibitors in lowering intraocular pressure. See also WO 00/38667, WO 99/32441, WO 99/02165, WO 00/38663, WO 01/46140, EP 0855389, JP 2000-1472, US Patent No. 6,043,275 and WO 00/38690.

Prostaglandin and prostaglandin derivatives are known to lower intraocular pressure by increasing uveoscleral outflow. This is true for both the F type and A type of prostaglandins. This invention is particularly interested in those compounds that lower IOP via the uveoscleral outflow pathway and other mechanisms by which the E series prostaglandins (PGE<sub>2</sub>) may facilitate IOP reduction. The four recognized subtypes of the EP receptor are believed to modulate these effect of lowering IOP (EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>; *J. Lipid Mediators Cell Signaling*, Vol. 14, pages 83-87 (1996)). See also *J. Ocular Pharmacology*, Vol. 4, 1, pages 13-18 (1988); *J. Ocular Pharmacology and Therapeutics*, Vol. 11, 3, pages 447-454 (1995); *J. Lipid Mediators*, Vol. 6, pages 545-553 (1993); US Patent Nos. 5,698,598 and 5,462,968 and *Investigative Ophthalmology and Visual Science*, Vol. 31, 12, pages 2560-2567 (1990). Of particular interest to this invention are compounds, which are agonist of the EP<sub>4</sub> subtype receptor.

A problem with using prostaglandins or derivatives thereof to lower intraocular pressure is that these compounds often induce an initial increase in intraocular pressure, can change the color of eye pigmentation and cause proliferation of some tissues surrounding the eye.

As can be seen, there are several current therapies for treating glaucoma and elevated intraocular pressure, but the efficacy and the side effect profiles of these agents are not ideal. Therefore, there still exist the need for new and effective therapies with little or no side effects.

A variety of disorders in humans and other mammals involve or are associated with abnormal or excessive bone loss. Such disorders include, but are not

limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma. One of the most common of these disorders is osteoporosis, which in its most frequent manifestation occurs in postmenopausal women. Osteoporosis is a systemic skeletal disease characterized by a low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Osteoporotic fractures are a major cause of morbidity and mortality in the elderly population. As many as 50% of women and a third of men will experience an osteoporotic fracture. A large segment of the older population already has low bone density and a high risk of fractures. There is a significant need to both prevent and treat osteoporosis and other conditions associated with bone resorption. Because osteoporosis, as well as other disorders associated with bone loss, are generally chronic conditions, it is believed that appropriate therapy will typically require chronic treatment.

Two different types of cells called osteoblasts and osteoclasts are involved in the bone formation and resorption processes, respectively. See H. Fleisch, *Bisphosphonates In Bone Disease, From The Laboratory To The Patient*, 3rd Edition, Parthenon Publishing (1997), which is incorporated by reference herein in its entirety.

Osteoblasts are cells that are located on the bone surface. These cells secrete an osseous organic matrix, which then calcifies. Substances such as fluoride, parathyroid hormone, and certain cytokines such as prostaglandins are known to provide a stimulatory effect on osteoblast cells. However, an aim of current research is to develop therapeutic agents that will selectively increase or stimulate the bone formation activity of the osteoblasts.

Osteoclasts are usually large multinucleated cells that are situated either on the surface of the cortical or trabecular bone or within the cortical bone. The osteoclasts resorb bone in a closed, sealed-off microenvironment located between the cell and the bone. The recruitment and activity of osteoclasts is known to be influenced by a series of cytokines and hormones. It is well known that bisphosphonates are selective inhibitors of osteoclastic bone resorption, making these compounds important therapeutic agents in the treatment or prevention of a variety of systemic or localized bone

disorders caused by or associated with abnormal bone resorption. However, despite the utility of bisphosphonates there remains the desire amongst researchers to develop additional therapeutic agents for inhibiting the bone resorption activity of osteoclasts.

5 Prostaglandins such as the PGE<sub>2</sub> series are known to stimulate bone formation and increase bone mass in mammals, including man. It is believed that the four different receptor subtypes, designated EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub> are involved in mediating the bone modeling and remodeling processes of the osteoblasts and osteoclasts. The major prostaglandin receptor  
10 in bone is EP<sub>4</sub>, which is believed to provide its effect by signaling via cyclic AMP.

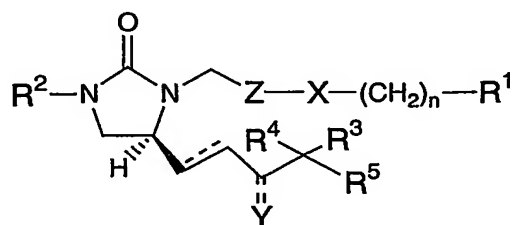
In present invention it is further found that the formula I agonists of the EP<sub>4</sub> subtype receptor are useful for stimulating bone formation.

15 WO 02/24647, WO 02/42268, EP 1114816, WO 01/46140 and WO 01/72268 disclose EP<sub>4</sub> agonists. However, they do not disclose the compounds of the instant invention.

#### SUMMARY OF THE INVENTION

20 This invention relates to potent selective agonists of the EP<sub>4</sub> subtype of prostaglandin E<sub>2</sub> receptors, their use or a formulation thereof in the treatment of glaucoma and other conditions that are related to elevated intraocular pressure in the eye of a patient. Another aspect of this invention relates to the use of such compounds to provide a neuroprotective effect to the eye of mammalian species, particularly humans. This invention further relates  
25 to the use of the compounds of this invention for mediating the bone modeling and remodeling processes of the osteoblasts and osteoclasts.

More particularly, this invention relates to novel EP<sub>4</sub> agonist having the structural formula I:



## FORMULA I

or a pharmaceutically acceptable salt, enantiomer, diastereomer, prodrug or mixture thereof, wherein,

5 X is a bond, O or S;

Y represents =O, or -OH;

10 R<sup>1</sup> represents hydroxy, CN, (CH<sub>2</sub>)<sub>p</sub>CO<sub>2</sub>R<sup>6</sup>, (CH<sub>2</sub>)<sub>n</sub>SO<sub>3</sub>R<sup>6</sup>, -CF<sub>2</sub>SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NH<sub>2</sub>, -CONHSO<sub>2</sub>R<sub>2</sub>, -SO<sub>2</sub>NHCOR<sub>2</sub>, -PO(OH)<sub>2</sub>, CONHPO<sub>2</sub>R<sup>6</sup>, CONHR<sup>8</sup>, C<sub>1-4</sub> alkoxy, -(CH<sub>2</sub>)<sub>n</sub>NR<sup>6</sup>R<sup>7</sup>, hydroxymethylketone or (CH<sub>2</sub>)<sub>n</sub>heterocyclyl, said heterocyclyl unsubstituted or substituted with 1 to 3 groups of R<sup>a</sup> and optionally containing an acidic hydrogen atom;

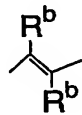
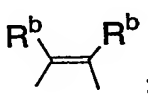
15 R<sup>2</sup> represents hydrogen, C<sub>6-10</sub> aryl, or C<sub>1-4</sub> alkyl;

R<sup>3</sup> and R<sup>4</sup> independently represents hydrogen, halogen, or C<sub>1-6</sub> alkyl;

20 R<sup>5</sup> independently represent (CH<sub>2</sub>)<sub>m</sub>C<sub>6-10</sub>aryl, (CH<sub>2</sub>)<sub>m</sub>C<sub>5-10</sub>heteroaryl, (CH<sub>2</sub>)<sub>m</sub>C<sub>3-10</sub> heterocycloalkyl, (CH<sub>2</sub>)<sub>m</sub>C<sub>3-8</sub> cycloalkyl said cycloalkyl, heterocycloalkyl, aryl or heteroaryl unsubstituted or substituted with 1-3 groups of R<sup>a</sup>;

R<sup>6</sup> and R<sup>7</sup> independently represents hydrogen, or C<sub>1-4</sub> alkyl;

25 R<sup>8</sup> represents hydrogen, acyl, or sulfonyl;

Z represents (C(R<sup>b</sup>)<sub>2</sub>)<sub>n</sub>,  or  ;

R<sup>b</sup> independently represents H, halogen, C<sub>1-6</sub> alkyl, C<sub>3-6</sub> cylcoalkyl;

30

R<sup>a</sup> represents C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> alkyl, CF<sub>3</sub>, nitro, amino, cyano, C<sub>1-6</sub> alkylamino, or halogen;

--- represents a double or single bond;

p represents 1-3;

5 n represents 0-4; and

m represents 0-8.

This and other aspects of the invention will be realized upon  
10 inspection of the invention as a whole.

#### DETAILED DESCRIPTION OF THE INVENTION

The invention is described herein in detail using the terms defined below unless otherwise specified.

15 The term "therapeutically effective amount", as used herein, means that amount of the EP<sub>4</sub> receptor subtype agonist of formula I, or other actives of the present invention, that will elicit the desired therapeutic effect or response or provide the desired benefit when administered in accordance with the desired treatment regimen. A preferred therapeutically effective amount relating to the treatment of  
20 abnormal bone resorption is a bone formation, stimulating amount. Likewise, a preferred therapeutically effective amount relating to the treatment of ocular hypertension or glaucoma is an amount effective for reducing intraocular pressure and/or treating ocular hypertension and/or glaucoma.

"Pharmaceutically acceptable" as used herein, means generally  
25 suitable for administration to a mammal, including humans, from a toxicity or safety standpoint.

The term "prodrug" refers to compounds which are drug precursors which, following administration and absorption, release the claimed drug in vivo via some metabolic process. A non-limiting example of a prodrug  
30 of the compounds of this invention would be an acid of the pyrrolidinone group, where the acid functionality has a structure that makes it easily hydrolyzed after administration to a patient. Exemplary prodrugs include acetic acid derivatives that are non-narcotic, analgesics/non-steroidal, anti-inflammatory drugs having a free CH<sub>2</sub>COOH group (which can optionally be  
35 in the form of a pharmaceutically acceptable salt, e.g. -CH<sub>2</sub>COO-Na+),

typically attached to a ring system, preferably to an aromatic or heteroaromatic ring system.

The term "alkyl" refers to a monovalent alkane (hydrocarbon) derived radical containing from 1 to 10 carbon atoms unless otherwise defined.

5 It may be straight, branched or cyclic. Preferred alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, cyclopentyl and cyclohexyl. When the alkyl group is said to be substituted with an alkyl group, this is used interchangeably with "branched alkyl group".

Cycloalkyl is a specie of alkyl containing from 3 to 15 carbon atoms, without alternating or resonating double bonds between carbon atoms. It may contain from 1 to 4 rings, which are fused. Examples of cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

Alkoxy refers to C<sub>1</sub>-C<sub>6</sub> alkyl-O-, with the alkyl group optionally substituted as described herein. Examples of alkoxy groups are methoxy, ethoxy, propoxy, butoxy and isomeric groups thereof.

Halogen (halo) refers to chlorine, fluorine, iodine or bromine.

Aryl refers to aromatic rings e.g., phenyl, substituted phenyl and the like, as well as rings which are fused, e.g., naphthyl, phenanthrenyl and the like. An aryl group thus contains at least one ring having at least 6 atoms, with up to five such rings being present, containing up to 22 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms or suitable heteroatoms. The preferred aryl groups are phenyl, naphthyl and phenanthrenyl. Aryl groups may likewise be substituted as defined. Preferred substituted aryls include phenyl and naphthyl.

25 The term "heterocycloalkyl" refers to a cycloalkyl group (nonaromatic) having 3 to 10 carbon atoms in which one of the carbon atoms in the ring is replaced by a heteroatom selected from O, S or N, and in which up to three additional carbon atoms may be replaced by hetero atoms.

The term "cycloalkyl" refers to a cyclic alkyl group (nonaromatic) having 3 to 10 carbon atoms.

The term "heteroatom" means O, S or N, selected on an independent basis.

The term "heteroaryl" refers to a monocyclic aromatic hydrocarbon group having 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing at least one heteroatom, O, S or N, in which a

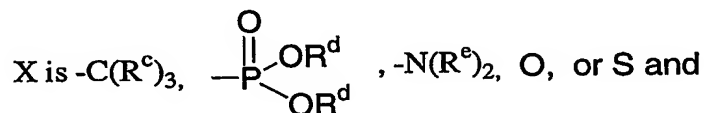
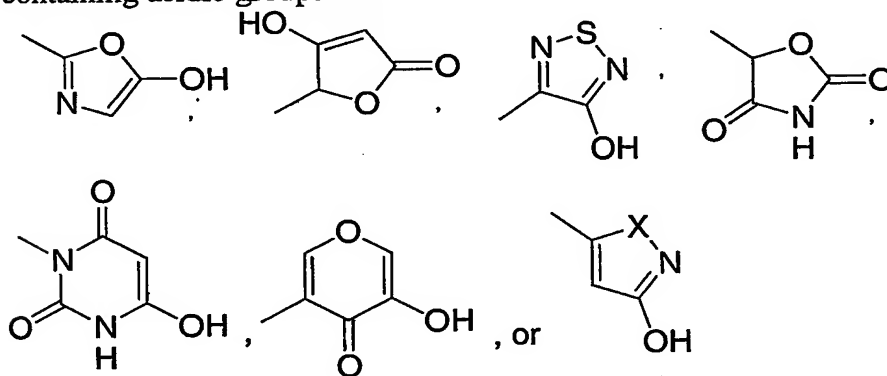
carbon or nitrogen atom is the point of attachment, and in which one or two additional carbon atoms is optionally replaced by a heteroatom selected from O or S, and in which from 1 to 3 additional carbon atoms are optionally replaced by nitrogen heteroatoms, said heteroaryl group being optionally substituted as described herein. Examples of this type are pyrrole, pyridine, oxazole, thiazole, tetrazole, and oxazine. For purposes of this invention the tetrazole includes all tautomeric forms. Additional nitrogen atoms may be present together with the first nitrogen and oxygen or sulfur, giving, e.g., thiadiazole.

The term heterocyclyl or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring. The term heterocycle or heterocyclic includes heteroaryl moieties. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, 1,3-dioxolanyl, furyl, imidazolidinyl, imidazoliny, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl. An embodiment of the examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl,



dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl,  
 imidazolidinyl, imidazolyl, imidazolyl, indolinyl, indolyl, isochromanyl,  
 isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl,  
 morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-  
 5 oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl,  
 pyridyl, 2-pyridinonyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl,  
 pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalyl,  
 tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl,  
 thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl,  
 10 thienyl and triazolyl.

For purposes of this invention, heterocyclyls containing acidic groups are those heterocyclyl groups that have an acidic hydrogen atom and can have a pKa in the range of 3 to 7. Non-limiting examples of heterocyclyls containing acidic groups are:



each  $R^c$  independently is H, fluorine,  
 cyano or  $C_{1-4}$  alkyl;  
 each  $R^d$  independently is H,  $C_{1-4}$  alkyl,  
 or a pharmaceutically acceptable cation;  
 each  $R^e$  independently is H,  $-C(=O)-R^f$ ,  
 or  $-SO_2R^e$ , wherein  $R^f$  is  $C_{1-4}$  linear alkyl  
 or phenyl

The term "agonist" as used herein means EP4 subtype compounds of formula I interact with the EP4 receptor to produce maximal, super maximal or submaximal effects compared to the natural agonist, PGE2. See Goodman and Gilman, The Pharmacological Basis of Therapeutics, 9<sup>th</sup> edition, 1996, chapter 2.

One embodiment of this invention is realized when R<sup>1</sup> is CN, (CH<sub>2</sub>)<sub>m</sub>C<sub>5-10</sub>heterocyclyl, -PO(OH)<sub>2</sub>, CONHPO<sub>2</sub>R<sup>6</sup>, (CH<sub>2</sub>)<sub>p</sub>CO<sub>2</sub>R<sup>6</sup>, CONHR<sup>6</sup> said heterocyclyl unsubstituted or substituted with 1 to 3 groups of R<sup>a</sup> and all other variables are as originally described. A subembodiment of this invention is realized when X is a bond. Another subembodiment of this invention is when X is Sulfur. When R<sup>1</sup> is (CH<sub>2</sub>)<sub>p</sub>CO<sub>2</sub>R<sup>6</sup>, and X is sulfur, the sulfur is divalent. Another embodiment of this invention is when X is O.

Another embodiment of this invention is realized when R<sup>1</sup> is (CH<sub>2</sub>)<sub>m</sub>C<sub>5-10</sub>heterocyclyl, said heterocyclyl unsubstituted or substituted with 1 to 3 groups of R<sup>a</sup> and all other variables are as originally described. A subembodiment of this invention is realized when X is a bond. Another subembodiment of this invention is realized when X is S. Another embodiment of this invention is when X is O.

Another embodiment of this invention is realized when R<sup>5</sup> is (CH<sub>2</sub>)<sub>m</sub>C<sub>6-10</sub>aryl, said aryl unsubstituted or substituted with 1 to 3 groups of R<sup>a</sup> and all other variables are as originally described.

A sub-embodiment of this invention is realized when R<sup>1</sup> is COOR<sup>6</sup>, CONHR<sup>6</sup>, -PO(OH)<sub>2</sub>, CONHPO<sub>2</sub>R<sup>6</sup> or tetrazolyl said tetrazolyl unsubstituted or substituted with a R<sup>a</sup> group and all other variables are as originally described. and all other variables are as originally described. A subembodiment of this invention is realized when X is a bond. Another subembodiment of this invention is realized when X is S. Another embodiment of this invention is when X is O.

Still another embodiment of this invention is realized when R<sup>5</sup> is a phenyl unsubstituted or substituted with 1 to 3 groups of R<sup>a</sup> and all other variables are as originally described.

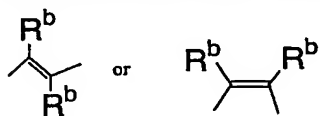
Yet another embodiment of this invention is realized when R<sup>1</sup> is tetrazolyl and R<sup>5</sup> is phenyl, said tetrazolyl unsubstituted or substituted with a R<sup>a</sup> group and phenyl is unsubstituted or substituted with 1-3 groups of R<sup>a</sup>, and

all other variables are as originally described. A subembodiment of this invention is realized when X is a bond. Another subembodiment of this invention is realized when X is S. Another embodiment of this invention is when X is O.

5 Still another embodiment of this invention is realized when  $\text{==Y}$  is  $\text{-OH}$ .

Still another embodiment of this invention is realized when  $\text{==Y}$  is  $\text{=O}$ .

10 Still another embodiment of this invention is realized when Z represents  $(\text{CH}_2)_n$ ,  $\text{C(halo)}_2$  or a double bond described by



and all other variables are as originally described. A subembodiment of this invention is realized when X is a bond. Another subembodiment is realized when X is S. Still another subembodiment is realized when X is O

15

Compounds of this invention are:

(4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-[6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,

20 (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-[6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,

(5*S*)-1-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,

(4*S*)-3-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methylimidazolidin-2-one,

25 (4*S*)-1-benzyl-3-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,

(5*S*)-1-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,

(4*S*)-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methylimidazolidin-2-one,

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(4*S*)-1-benzyl-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,

- (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-[4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl]imidazolidin-2-one,
- (4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-{4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl}imidazolidin-2-one,
- 5 (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-{4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl}imidazolidin-2-one,
- (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,
- (4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,
- 10 (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,
- (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-{4-[1-(1*H*-tetraazol-5-ylmethyl)cyclopropyl]butyl}imidazolidin-2-one,
- 15 (4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-{4-[1-(1*H*-tetraazol-5-ylmethyl)cyclopropyl]butyl}imidazolidin-2-one,
- (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-{4-[1-(1*H*-tetraazol-5-ylmethyl)cyclopropyl]butyl}imidazolidin-2-one,
- (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-(3-{1-[2-(1*H*-tetraazol-5-yl)ethyl]cyclopropyl}propyl)imidazolidin-2-one,
- 20 (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-[6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one
- (4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-(3-{1-[2-(1*H*-tetraazol-5-yl)ethyl]cyclopropyl}propyl)imidazolidin-2-one,
- 25 (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-(3-{1-[2-(1*H*-tetraazol-5-yl)ethyl]cyclopropyl}propyl)imidazolidin-2-one,
- (5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-(2-{1-[3-(1*H*-tetraazol-5-yl)propyl]cyclopropyl}ethyl)imidazolidin-2-one,
- (4*S*)-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-(2-{1-[3-(1*H*-tetraazol-5-yl)propyl]cyclopropyl}ethyl)imidazolidin-2-one,
- 30 (4*S*)-1-benzyl-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-(2-{1-[3-(1*H*-tetraazol-5-yl)propyl]cyclopropyl}ethyl)imidazolidin-2-one,
- (5*S*)-1-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,

- (4*S*)-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-1-methylimidazolidin-2-one,  
(4*S*)-1-benzyl-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-4-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]imidazolidin-2-one,  
5 (5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-{4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl}imidazolidin-2-one,  
(4*S*)-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-{4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl}imidazolidin-2-one,  
(4*S*)-1-benzyl-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-{4-[(1*H*-tetraazol-5-ylmethyl)thio]butyl}imidazolidin-2-one,  
10 (5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,  
(4*S*)-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-methyl-3-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,  
15 (4*S*)-1-benzyl-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-[4-(1*H*-tetraazol-5-ylmethoxy)butyl]imidazolidin-2-one,  
(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,  
(4*S*)-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-1-methylimidazolidin-2-one,  
20 (4*S*)-1-benzyl-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-[4,4-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,  
(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-1-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,  
25 (4*S*)-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]-1-methylimidazolidin-2-one,  
(4*S*)-1-benzyl-4-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-[3,3-difluoro-6-(1*H*-tetraazol-5-yl)hexyl]imidazolidin-2-one,  
7-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}heptanoic acid,  
30 7-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}heptanoic acid.  
7-{(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}heptanoic acid,

methyl-7-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)heptanoate,  
methyl-7-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl)heptanoate,  
5 methyl-7-((5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)heptanoate,  
[(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butyl)thio]acetic acid,  
[(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl)butyl)thio]acetic acid,  
10 [(4-((5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butyl)thio]acetic acid,  
methyl-[(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butyl)thio]acetate,  
15 methyl-[(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl)butyl)thio]acetate,  
methyl-[(4-((5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butyl)thio]acetate,  
(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butoxy)acetic acid,  
20 (4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl)butoxy)acetic acid,  
(4-((5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butoxy)acetic acid,  
25 methyl-(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butoxy)acetate,  
methyl-(4-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl)butoxy)acetate,  
methyl-(4-((5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)butoxy)acetate,  
30 7-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)heptanoic acid,  
methyl-7-((5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl)heptanoate,

- 7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]heptanoic acid,  
methyl-7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]heptanoate,
- 5 7-[(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]heptanoic acid,  
methyl-7-[(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]heptanoate,
- 10 7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoic acid,  
methyl-7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoate,
- 15 7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoic acid,  
methyl-7-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoate,
- 20 7-[(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoic acid,  
methyl-7-[(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]-4,4-difluoroheptanoate,
- 25 6-[(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]hexylphosphonic acid,  
6-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]hexylphosphonic acid,
- 30 6-[(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]hexylphosphonic acid,  
6-[(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl]hexylphosphonic acid,  
6-[(5*S*)-3-benzyl-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]hexylphosphonic acid,  
6-[(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]hexylphosphonic acid,  
[(4-[(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl]butyl)thio]methylphosphonic acid,

- [(4-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- [(4-{(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- 5 [(4-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- [(4-{(5*S*)-3-benzyl-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- [(4-{(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- 10 [(1,1-difluoro-4-{(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- 6-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}-3,3-difluorohexylphosphonic acid,
- 15 [(1,1-difluoro-4-{(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}butyl)thio]methylphosphonic acid,
- [(4-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}-1,1-difluorobutyl)thio]methylphosphonic acid,
- [(4-{(5*S*)-3-benzyl-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}-1,1-difluorobutyl)thio]methylphosphonic acid,
- 20 [(4-{(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}-1,1-difluorobutyl)thio]methylphosphonic acid,
- 2-[1-(3-{(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,
- 25 2-[1-(3-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,
- 2-[1-(3-{(5*S*)-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,
- 2-[1-(3-{(5*S*)-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,
- 30 2-[1-(3-{(5*S*)-3-benzyl-5-[(1*E*)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,
- 2-[1-(3-{(5*S*)-3-benzyl-5-[(1*E*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]ethylphosphonic acid,



N-{3-[1-(3-{(5S)-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}methanesulfonamide,

N-{3-[1-(3-{(5S)-5-[(1E)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}methanesulfonamide,

5 N-{3-[1-(3-{(5S)-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}methanesulfonamide,

N-{3-[1-(3-{(5S)-5-[(1E)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-3-methyl-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}-methanesulfonamide,

10 N-{3-[1-(3-{(5S)-3-benzyl-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}methanesulfonamide, or

N-{3-[1-(3-{(5S)-3-benzyl-5-[(1E)-4,4-difluoro-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidin-1-yl}propyl)cyclopropyl]propanoyl}-

methanesulfonamide or a pharmaceutically acceptable salt, enantiomer, 15 diastereomer, prodrug or mixture thereof.

Another embodiment of this invention is directed to a composition containing an EP<sub>4</sub> agonist of Formula I and optionally a pharmaceutically acceptable carrier.

Yet another embodiment of this invention is directed to a 20 method for decreasing elevated intraocular pressure or treating glaucoma by administration, preferably topical or intra-cameral administration, of a composition containing an EP<sub>4</sub> agonist of Formula I and optionally a pharmaceutically acceptable carrier. Use of the compounds of formula I for the manufacture of a medicament for treating elevated intraocular pressure or 25 glaucoma or a combination thereof is also included in this invention

This invention is further concerned with a process for making a pharmaceutical composition comprising a compound of formula I.

This invention is further concerned with a process for making a pharmaceutical composition comprising a compound of formula I, and a 30 pharmaceutically acceptable carrier.

The claimed compounds bind strongly and act on PGE<sub>2</sub> receptor, particularly on the EP<sub>4</sub> subtype receptor and therefore are useful for preventing and/or treating glaucoma and ocular hypertension.

Dry eye is a common ocular surface disease afflicting millions 35 of people. Although it appears that dry eye may result from a number of

unrelated pathogenic causes, the common end result is the breakdown of the tear film, which results in dehydration of the exposed outer surface of the eye. (Lemp, Report of the Nation Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes, The *CLAO Journal*, 21(4):221-231 (1995)). One cause for dry eye is the decreased mucin production by the conjunctival cells and/or corneal epithelial cells of mucin, which protects and lubricates the ocular surface (Gipson and Inatomi, Mucin genes expressed by ocular surface epithelium. *Progress in Retinal and Eye Research*, 16:81-98 (1997)). Functional EP4 receptors have been found in human conjunctival epithelial cells (see US Patent 6,344,477, incorporated by reference in its entirety) and it is appreciated that both human corneal epithelial cells (*Progress in Retinal and Eye Research*, 16:81-98(1997)) and conjunctival cells (Dartt et al. Localization of nerves adjacent to goblet cells in rat conjunctiva. *Current Eye Research*, 14:993-1000 (1995)) are capable of secreting mucins. Thus, the compounds of formula I are useful for treating dry eye.

Macular edema is swelling within the retina within the critically important central visual zone at the posterior pole of the eye. An accumulation of fluid within the retina tends to detach the neural elements from one another and from their local blood supply, creating a dormancy of visual function in the area. It is believed that EP4 agonist which lower IOP are useful for treating diseases of the macular such as macular edema or macular degeneration. Thus, another aspect of this invention is a method for treating macular edema or macular degeneration.

Glaucoma is characterized by progressive atrophy of the optic nerve and is frequently associated with elevated intraocular pressure (IOP). It is possible to treat glaucoma, however, without necessarily affecting IOP by using drugs that impart a neuroprotective effect. See *Arch. Ophthalmol.* Vol. 112, Jan 1994, pp. 37-44; *Investigative Ophthalmol. & Visual Science*, 32, 5, April 1991, pp. 1593-99. It is believed that EP4 agonist which lower IOP are useful for providing a neuroprotective effect. They are also believed to be effective for increasing retinal and optic nerve head blood velocity and increasing retinal and optic nerve oxygen by lowering IOP, which when coupled together benefits optic nerve health. As a result, this invention further relates to a method for increasing retinal and optic nerve head blood velocity, or increasing retinal and optic nerve oxygen tension or providing a

neuroprotective effect or a combination thereof by using an EP4 agonist of formula I.

The compounds produced in the present invention are readily combined with suitable and known pharmaceutically acceptable excipients to produce compositions which may be administered to mammals, including humans, to achieve effective IOP lowering. Thus, this invention is also concerned with a method of treating ocular hypertension or glaucoma by administering to a patient in need thereof one of the compounds of formula I alone or in combination with a  $\beta$ -adrenergic blocking agent such as timolol, betaxolol, levobetaxolol, carteolol, levobunolol, a parasympathomimetic agent such as pilocarpine, a sympathomimetic agents such as epinephrine, iopidine, brimonidine, clonidine, para-aminoclonidine, a carbonic anhydrase inhibitor such as dorzolamide, acetazolamide, metazolamide or brinzolamide; a Maxi-K channel blocker as disclosed in USSN 60/389,205, filed June 17, 2002 (Attorney Docket 21121PV), 60/389,222, filed June 17, 2002 (Attorney docket 21092PV), 60/458,981, filed March 27, 2003 (Attorney docket 21101PV4), 60/424790, filed November 8, 2002 (Attorney docket 21260PV), 60/424808, filed November 8, 2002 (Attorney docket 21281PV), 09/765716, filed January 17, 2001, 09/764738, filed January 17, 2001 and PCT publications WO 02/077168 and WO 02/02060863, all incorporated by reference in their entirety herein, a prostaglandin such as latanoprost, travaprost, unoprostone, rescula, S1033 (compounds set forth in US Patent Nos. 5,889,052; 5,296,504; 5,422,368; and 5,151,444); a hypotensive lipid such as lumigan and the compounds set forth in US Patent No. 5,352,708; a neuroprotectant disclosed in US Patent No. 4,690,931, particularly eliprodil and R-eliprodil as set forth in WO 94/13275, including memantine; or an agonist of 5-HT<sub>2</sub> receptors as set forth in PCT/US00/31247, particularly 1-(2-aminopropyl)-3-methyl-1H-imidazol-6-ol fumarate and 2-(3-chloro-6-methoxy-indazol-1-yl)-1-methyl-ethylamine.

Thus, this invention is also concerned with a method for increasing retinal and optic nerve head blood velocity, or increasing retinal and optic nerve oxygen tension or providing a neuroprotective effect or a combination thereof by administering to a patient in need thereof one of the compounds of formula I alone or in combination with a  $\beta$ -adrenergic blocking

agent such as timolol, betaxolol, levobetaxolol, carteolol, levobunolol, a  
parasympathomimetic agent such as pilocarpine, a sympathomimetic agents  
such as epinephrine, iopidine, brimonidine, clonidine, para-aminoclonidine, a  
carbonic anhydrase inhibitor such as dorzolamide, acetazolamide,  
5 metazolamide or brinzolamide; a prostaglandin such as latanoprost, travaprost,  
unoprostone, rescula, S1033 (compounds set forth in US Patent Nos.  
5,889,052; 5,296,504; 5,422,368; and 5,151,444); a Maxi-K channel blocker as  
disclosed in USSN 60/389,205, filed June 17, 2002 (Attorney Docket  
21121PV), 60/389,222, filed June 17, 2002 (Attorney docket 21092PV),  
10 60/458,981, filed March 27, 2003 (Attorney docket 21101PV4), 60/424790,  
filed November 8, 2002 (Attorney docket 21260PV), 60/424808, filed  
November 8, 2002 (Attorney docket 21281PV), 09/765716, filed January 17,  
2001, 09/764738, filed January 17, 2001 and PCT publications WO 02/077168  
and WO 02/02060863, all incorporated by reference in their entirety herein, a  
15 hypotensive lipid such as lumigan and the compounds set forth in US Patent  
No. 5,352,708; a neuroprotectant disclosed in US Patent No. 4,690,931,  
particularly eliprodil and R-eliprodil as set forth in WO 94/13275, including  
memantine; or an agonist of 5-HT<sub>2</sub> receptors as set forth in PCT/US00/31247,  
particularly 1-(2-aminopropyl)-3-methyl-1H-imidazol-6-ol fumarate and 2-(3-  
20 chloro-6-methoxy-indazol-1-yl)-1-methyl-ethylamine. Use of the compounds  
of formula I for the manufacture of a medicament for increasing retinal and  
optic nerve head blood velocity, or increasing retinal and optic nerve oxygen  
tension or providing a neuroprotective effect or a combination thereof is also  
included in this invention.

25               This invention is further concerned with a method for treating  
macular edema or macular degeneration by administering to a patient in need  
thereof one of the compounds of formula I alone or in combination with a  $\beta$ -  
adrenergic blocking agent such as timolol, betaxolol, levobetaxolol, carteolol,  
levobunolol, a parasympathomimetic agent such as pilocarpine, a  
30 sympathomimetic agents such as epinephrine, iopidine, brimonidine, clonidine,  
para-aminoclonidine, a carbonic anhydrase inhibitor such as dorzolamide,  
acetazolamide, metazolamide or brinzolamide; a Maxi-K channel blocker as  
disclosed in USSN 60/389,205, filed June 17, 2002 (Attorney Docket  
21121PV), 60/389,222, filed June 17, 2002 (Attorney docket 21092PV),  
35 60/458,981, filed March 27, 2003 (Attorney docket 21101PV4), 60/424790,

filed November 8, 2002 (Attorney docket 21260PV), 60/424808, filed  
November 8, 2002 (Attorney docket 21281PV), 09/765716, filed January 17,  
2001, 09/764738, filed January 17, 2001 and PCT publications WO 02/077168  
and WO 02/02060863, all incorporated by reference in their entirety herein, a  
5 prostaglandin such as latanoprost, travaprost, unoprostone, rescula, S1033  
(compounds set forth in US Patent Nos. 5,889,052; 5,296,504; 5,422,368; and  
5,151,444); a hypotensive lipid such as lumigan and the compounds set forth in  
US Patent No. 5,352,708; a neuroprotectant disclosed in US Patent No.  
4,690,931, particularly eliprodil and R-eliprodil as set forth in WO 94/13275,  
10 including memantine; or an agonist of 5-HT<sub>2</sub> receptors as set forth in  
PCT/US00/31247, particularly 1-(2-aminopropyl)-3-methyl-1H-imidazol-6-ol  
fumarate and 2-(3-chloro-6-methoxy-indazol-1-yl)-1-methyl-ethylamine. Use  
of the compounds of formula I for the manufacture of a medicament for  
macular edema or macular degeneration is also included in this invention.

15           The EP<sub>4</sub> agonist used in the instant invention can be  
administered in a therapeutically effective amount intravaneously,  
subcutaneously, topically, transdermally, parenterally or any other method  
known to those skilled in the art. Ophthalmic pharmaceutical compositions are  
preferably adapted for topical administration to the eye in the form of solutions,  
20 suspensions, ointments, creams or as a solid insert. Ophthalmic formulations  
of this compound may contain from 0.001 to 5% and especially 0.001 to 0.1%  
of medicament. Higher dosages as, for example, up to about 10% or lower  
dosages can be employed provided the dose is effective in reducing intraocular  
pressure, treating glaucoma, increasing blood flow velocity or oxygen tension.  
25 For a single dose, from between 0.001 to 5.0 mg, preferably 0.005 to 2.0 mg,  
and especially 0.005 to 1.0 mg of the compound can be applied to the human  
eye.

          The pharmaceutical preparation which contains the compound  
may be conveniently admixed with a non-toxic pharmaceutical organic carrier,  
30 or with a non-toxic pharmaceutical inorganic carrier. Typical of  
pharmaceutically acceptable carriers are, for example, water, mixtures of water  
and water-miscible solvents such as lower alkanols or aralkanols, vegetable  
oils, peanut oil, polyalkylene glycols, petroleum based jelly, ethyl cellulose,  
ethyl oleate, carboxymethyl-cellulose, polyvinylpyrrolidone, isopropyl  
35 myristate and other conventionally employed acceptable carriers. The

pharmaceutical preparation may also contain non-toxic auxiliary substances such as emulsifying, preserving, wetting agents, bodying agents and the like, as for example, polyethylene glycols 200, 300, 400 and 600, carbowaxes 1,000, 1,500, 4,000, 6,000 and 10,000, antibacterial components such as quaternary ammonium compounds, phenylmercuric salts known to have cold sterilizing properties and which are non-injurious in use, thimerosal, methyl and propyl paraben, benzyl alcohol, phenyl ethanol, buffering ingredients such as sodium borate, sodium acetates, gluconate buffers, and other conventional ingredients such as sorbitan monolaurate, triethanolamine, oleate, polyoxyethylene sorbitan monopalmitate, dioctyl sodium sulfosuccinate, monothioglycerol, thiosorbitol, ethylenediamine tetracetic acid, and the like. Additionally, suitable ophthalmic vehicles can be used as carrier media for the present purpose including conventional phosphate buffer vehicle systems, isotonic boric acid vehicles, isotonic sodium chloride vehicles, isotonic sodium borate vehicles and the like. The pharmaceutical preparation may also be in the form of a microparticle formulation. The pharmaceutical preparation may also be in the form of a solid insert. For example, one may use a solid water soluble polymer as the carrier for the medicament. The polymer used to form the insert may be any water soluble non-toxic polymer, for example, cellulose derivatives such as methylcellulose, sodium carboxymethyl cellulose, (hydroxyloweralkyl cellulose), hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose; acrylates such as polyacrylic acid salts, ethylacrylates, polyacrylamides; natural products such as gelatin, alginates, pectins, tragacanth, karaya, chondrus, agar, acacia; the starch derivatives such as starch acetate, hydroxymethyl starch ethers, hydroxypropyl starch, as well as other synthetic derivatives such as polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyl methyl ether, polyethylene oxide, neutralized carbopol and xanthan gum, gellan gum, and mixtures of said polymer.

Suitable subjects for the administration of the formulation of the present invention include primates, man and other animals, particularly man and domesticated animals such as cats, rabbits and dogs.

The pharmaceutical preparation may contain non-toxic auxiliary substances such as antibacterial components which are non-injurious in use, for example, thimerosal, benzalkonium chloride, methyl and propyl paraben, benzyl dodecinium bromide, benzyl alcohol, or phenylethanol; buffering

ingredients such as sodium chloride, sodium borate, sodium acetate, sodium citrate, or gluconate buffers; and other conventional ingredients such as sorbitan monolaurate, triethanolamine, polyoxyethylene sorbitan monopalmitate, ethylenediamine tetraacetic acid, and the like.

5           The ophthalmic solution or suspension may be administered as often as necessary to maintain an acceptable IOP level in the eye. It is contemplated that administration to the mammalian eye will be from once up to three times daily.

10           For topical ocular administration the novel formulations of this invention may take the form of solutions, gels, ointments, suspensions or solid inserts, formulated so that a unit dosage comprises a therapeutically effective amount of the active component or some multiple thereof in the case of a combination therapy.

15           The compounds of the instant invention are also useful for mediating the bone modeling and remodeling processes of the osteoblasts and osteoclasts. See PCT US99/23757 filed October 12, 1999 and incorporated herein by reference in its entirety. The major prostaglandin receptor in bone is EP<sub>4</sub>, which is believed to provide its effect by signaling via cyclic AMP. See Ikeda T, Miyaura C, Ichikawa A, Narumiya S, Yoshiki S and Suda T 1995, In situ localization of three subtypes (EP<sub>1</sub>, EP<sub>3</sub> and EP<sub>4</sub>) of prostaglandin E  
20           receptors in embryonic and newborn mice., *J Bone Miner Res* **10** (sup 1):S172, which is incorporated by reference herein in its entirety. Use of the compounds of formula I for the manufacture of a medicament for mediating the bone modeling and remodeling processes are also included in this invention.

25           Thus, another object of the present invention is to provide methods for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP<sub>4</sub> receptor subtype agonist of formula I.

30           Still another object of the present invention to provide methods for stimulating bone formation in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of an EP<sub>4</sub> receptor subtype agonist of formula I and a bisphosphonate active. Use of the compounds of formula I for the manufacture of a medicament for stimulating bone formation is also included in this invention.

Yet another object of the present invention to provide pharmaceutical compositions comprising a therapeutically effective amount of an EP<sub>4</sub> receptor subtype agonist of formula I and a bisphosphonate active.

It is another object of the present invention to provide methods  
5 for treating or reducing the risk of contracting a disease state or condition related to abnormal bone resorption in a mammal in need of such treatment or prevention, comprising administering to said mammal a therapeutically effective amount of an EP<sub>4</sub> receptor subtype agonist of formula I. Use of the compounds of formula I for the manufacture of a medicament for treating or  
10 reducing the risk of contracting a disease state or condition related to abnormal bone resorption is also included in this invention.

The disease states or conditions related to abnormal bone resorption include, but are not limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss,  
15 bone fractures, rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.

Within the method comprising administering a therapeutically effective amount of an EP<sub>4</sub> receptor subtype agonist of formula I and a bisphosphonate active, both concurrent and sequential administration of the EP<sub>4</sub> receptor subtype agonist of  
20 formula I and the bisphosphonate active are deemed within the scope of the present invention. Generally, the formulations are prepared containing 5 or 10 mg of a bisphosphonate active, on a bisphosphonic acid active basis. With sequential administration, the agonist and the bisphosphonate can be administered in either order. In a subclass of sequential administration the agonist and bisphosphonate are  
25 typically administered within the same 24 hour period. In yet a further subclass, the agonist and bisphosphonate are typically administered within about 4 hours of each other.

Nonlimiting examples of bisphosphonate actives useful herein include the following:

30 Alendronic acid, 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid;

Alendronate (also known as alendronate sodium or alendronate monosodium trihydrate), 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid monosodium trihydrate;



Alendronic acid and alendronate are described in U.S. Patents 4,922,007, to Kieczkowski et al., issued May 1, 1990; 5,019,651, to Kieczkowski et al., issued May 28, 1991; 5,510,517, to Dauer et al., issued April 23, 1996; 5,648,491, to Dauer et al., issued July 15, 1997, all of which are  
5 incorporated by reference herein in their entirety;

Cycloheptylaminomethylene-1,1-bisphosphonic acid, YM 175, Yamanouchi (cimadronate), as described in U.S. Patent 4,970,335, to Isomura et al., issued November 13, 1990, which is incorporated by reference herein in its entirety;

10 1,1-dichloromethylene-1,1-diphosphonic acid (clodronic acid), and the disodium salt (clodronate, Procter and Gamble), are described in Belgium Patent 672,205 (1966) and *J. Org. Chem* 32, 4111 (1967), both of which are incorporated by reference herein in their entirety;

15 1-hydroxy-3-(1-pyrrolidinyl)-propylidene-1,1-bisphosphonic acid (EB-1053);

1-hydroxyethane-1,1-diphosphonic acid (etidronic acid);

1-hydroxy-3-(N-methyl-N-pentylamino)propylidene-1,1-bisphosphonic acid, also known as BM-210955, Boehringer-Mannheim (ibandronate), is described in U.S. Patent No. 4,927,814, issued May 22, 1990,  
20 which is incorporated by reference herein in its entirety;

6-amino-1-hydroxyhexylidene-1,1-bisphosphonic acid (neridronate);

3-(dimethylamino)-1-hydroxypropylidene-1,1-bisphosphonic acid (olpadronate);

25 3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid (pamidronate);

[2-(2-pyridinyl)ethylidene]-1,1-bisphosphonic acid (piridronate) is described in U.S. Patent No. 4,761,406, which is incorporated by reference in its entirety;

30 1-hydroxy-2-(3-pyridinyl)-ethylidene-1,1-bisphosphonic acid (risedronate);

(4-chlorophenyl)thiomethane-1,1-disphosphonic acid (tiludronate) as described in U.S. Patent 4,876,248, to Breliere et al., October 24, 1989, which is incorporated by reference herein in its entirety; and

1-hydroxy-2-(1H-imidazol-1-yl)ethylidene-1,1-bisphosphonic acid  
(zolendronate).

A non-limiting class of bisphosphonate actives useful in the  
instant invention are selected from the group consisting of alendronate,  
5 cimidronate, clodronate, tiludronate, etidronate, ibandronate, neridronate,  
olpandronate, risedronate, piridronate, pamidronate, zolendronate,  
pharmaceutically acceptable salts thereof, and mixtures thereof.

A non-limiting subclass of the above-mentioned class in the  
instant case is selected from the group consisting of alendronate,  
10 pharmaceutically acceptable salts thereof, and mixtures thereof.

A non-limiting example of the subclass is alendronate monosodium  
trihydrate.

In the present invention, as it relates to bone stimulation, the agonist is  
typically administered for a sufficient period of time until the desired therapeutic  
15 effect is achieved. The term "until the desired therapeutic effect is achieved", as used  
herein, means that the therapeutic agent or agents are continuously administered,  
according to the dosing schedule chosen, up to the time that the clinical or medical  
effect sought for the disease or condition being mediated is observed by the clinician  
or researcher. For methods of treatment of the present invention, the compounds are  
20 continuously administered until the desired change in bone mass or structure is  
observed. In such instances, achieving an increase in bone mass or a replacement of  
abnormal bone structure with normal bone structure are the desired objectives. For  
methods of reducing the risk of a disease state or condition, the compounds are  
continuously administered for as long as necessary to prevent the undesired condition.  
25 In such instances, maintenance of bone mass density is often the objective.

Nonlimiting examples of administration periods can range from about  
2 weeks to the remaining lifespan of the mammal. For humans, administration  
periods can range from about 2 weeks to the remaining lifespan of the human,  
preferably from about 2 weeks to about 20 years, more preferably from about 1 month  
30 to about 20 years, more preferably from about 6 months to about 10 years, and most  
preferably from about 1 year to about 10 years.

The instant compounds are also useful in combination with known  
agents useful for treating or preventing bone loss, bone fractures, osteoporosis,  
glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone  
35 turnover, periodontal disease, tooth loss, osteoarthritis, rheumatoid arthritis,

periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma. Combinations of the presently disclosed compounds with other agents useful in treating or preventing osteoporosis or other bone disorders are within the scope of the invention. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the disease involved. Such agents include the following: an organic bisphosphonate; a cathepsin K inhibitor; an estrogen or an estrogen receptor modulator; an androgen receptor modulator; an inhibitor of osteoclast proton ATPase; an inhibitor of HMG-CoA reductase; an integrin receptor antagonist; an osteoblast anabolic agent, such as PTH; calcitonin; Vitamin D or a synthetic Vitamin D analogue; and the pharmaceutically acceptable salts and mixtures thereof. A preferred combination is a compound of the present invention and an organic bisphosphonate. Another preferred combination is a compound of the present invention and an estrogen receptor modulator. Another preferred combination is a compound of the present invention and an estrogen. Another preferred combination is a compound of the present invention and an androgen receptor modulator. Another preferred combination is a compound of the present invention and an osteoblast anabolic agent.

Regarding treatment of abnormal bone resorption and ocular disorders, the formula I agonists generally have an EC<sub>50</sub> value from about 0.001 nM to about 100 microM, although agonists with activities outside this range can be useful depending upon the dosage and route of administration. In a subclass of the present invention, the agonists have an EC<sub>50</sub> value of from about 1 nM to about 10 microM. In a further subclass of the present invention, the agonists have an EC<sub>50</sub> value of from about 0.1 microM to about 10 microM. EC<sub>50</sub> is a common measure of agonist activity well known to those of ordinary skill in the art and is defined as the concentration or dose of an agonist that is needed to produce half, i.e. 50%, of the maximal effect. See also, Goodman and Gilman's, *The Pharmacologic Basis of Therapeutics*, 9th edition, 1996, chapter 2, E. M. Ross, *Pharmacodynamics, Mechanisms of Drug Action and the Relationship Between Drug Concentration and Effect*, and PCT US99/23757, filed October 12, 1999, which are incorporated by reference herein in their entirety.

The herein examples illustrate but do not limit the claimed invention. Each of the claimed compounds are EP4 agonists and are useful for a number of physiological ocular and bone disorders.

5 The compounds of this invention can be made, with some modification, in accordance with US Patent No. 6,043,275, EP0855389, USSN 60/337228 (Merck Docket No. MC052) and WO 01/46140, all of which are incorporated herein by reference in their entirety. The following non-limiting examples, given by way of illustration, is demonstrative of the present invention.

10

#### PREPARATION 1

1-benzyl 5-methyl (5R)-2-oxoimidazolidine-1,5-dicarboxylate

15

Step A: (4R)-3-[(benzyloxy)carbonyl]-2-oxoimidazolidine-4-carboxylic acid.

20

To a solution of NaOH (2.48 g, 61.97 mmole) in water (50 ml) at 0°C is added bromine (3.305 g, 20.66 mmole). After 5 min, (R)-NCbz-Asparagine is added to the above solution and the mixture is stirred at 50°C for 1h. Addition of 5% Na2S2O3 and then extraction with Et2O (1x100 ml). The aqueous phase is acidified to pH 2 with 6N HCl, and the reaction mixture is left in the fridge for 6 days. Filtration of the crystals, and recrystallization in hot water to afford (4R)-3-[(benzyloxy)carbonyl]-2-oxoimidazolidine-4-carboxylic acid as a white powder. <sup>1</sup>H NMR (DMSO-D6) δ 7.5-7.2 (M, 5 H), 6.5 (SL, 1H), 5.28 (S, 2H), 4.88 (M, 1H), 3.88 (M, 1H), 3.5 (M, 1H); MS 263.2 (M-1).

25

Step B: 1-benzyl 5-methyl (5R)-2-oxoimidazolidine-1,5-dicarboxylate.

To a solution of (4R)-3-[(benzyloxy)carbonyl]-2-oxoimidazolidine-4-carboxylic acid (1 g, 3.8 mmol) in MeOH (10 ml) at -20°C is added dropwise thionyl chloride (337 mg, 2.84 mmol). The mixture is stirred 2 h at -20°C, then overnight at rt. The solvent is removed under reduced pressure and the residue is dissolved in AcOEt, washed with sat. NaHCO<sub>3</sub> solution and dried on Na<sub>2</sub>SO<sub>4</sub> and the solvent removed. The residue is dissolved in a minimum of hot AcOEt, then cooled to rt and hexanes are added to crystallize 1-benzyl 5-methyl (5R)-2-oxoimidazolidine-1,5-dicarboxylate as a amorphous white powder. <sup>1</sup>H NMR (MeOH-D<sub>4</sub>) δ 7.5-7.2 (m, 5H), 6.4 (s, 1H), 5.38 (m, 1H), 5.2 (m, 1H), 4.8 (m, 1H), 3.80-3.65 (m, 4H), 3.45 (m, 1H); MS 279.1 (M+1).

Step C: 1-benzyl-4-methyl-(4R)-3-(6-cyanoheptyl)-2-oxoimidazolidine-1,4-dicarboxylate.

To a solution of 1-benzyl-5-methyl-(5R)-2-oxoimidazolidine-1,5-dicarboxylate (902 mg, 3.24 mmol) in DMF (8 ml) is added NaH (60% dispersion in oil, 136.3 mg, 3.40 mmol) at rt, and the mixture is stirred at rt for 30 min and at 50°C for 30 min. Then are added 7-bromoheptanenitrile (1.231 g, 6.48 mmol) and a catalytic amount of tetrabutylammonium iodide. The mixture is stirred at 50°C for 12 h and worked-up by addition of 1N HCl, extracted with AcOEt (3x 20 ml), the organic phases are washed with brine, dried on Na<sub>2</sub>SO<sub>4</sub> and the solvent removed. The residue is purified by flash chromatography on silica gel (1:1 AcOEt : Hexanes) to afford 1-benzyl-4-methyl-(4R)-3-(6-cyanoheptyl)-2-oxoimidazolidine-1,4-dicarboxylate as a oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.5-7.3 (m, 5H), 5.3 (s, 2H), 4.2 (m, 1H), 4.00 (m, 1H), 3.92 (m, 1H), 4.8 (s, 3H), 3.63 (m, 1H), 3.03 (m, 1H), 2.35 (m, 2H), 1.8-1.3 (m, 8H); MS 388.2 (M+1).

Step D: benzyl (4R)-3-(6-cyanoheptyl)-4-(hydroxymethyl)-2-oxoimidazolidine-1-carboxylate.

To a solution of 1-benzyl-4-methyl-(4R)-3-(6-cyanoheptyl)-2-oxoimidazolidine-1,4-dicarboxylate (1.424 g, 3.68 mmol) in MeOH (15 ml) at -20°C is added portionwise NaBH<sub>4</sub> (278 mg, 7.35 mmol) and the reaction mixture is allowed to reach rt and stirred for 2 h at rt. Addition of conc. HCl to

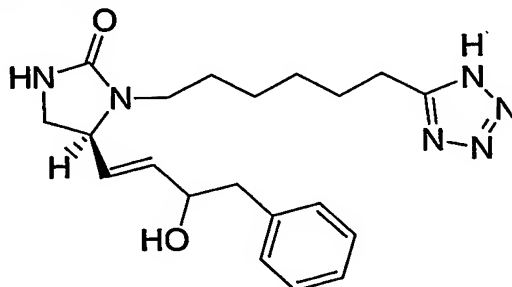
pH6 and the solvent is removed. The residue is dissolved in water and is extracted with AcOEt (3x 20 ml), the organic phases are washed with brine, dried on Na<sub>2</sub>SO<sub>4</sub> and the solvent removed. The residue is purified by flash chromatography on silica gel (AcOEt) to afford benzyl (4R)-3-(6-cyanohexyl)-4-(hydroxymethyl)-2-oxoimidazolidine-1-carboxylate as a oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.5-7.3 (M, 5H), 5.28 (M, 2H), 3.9 (M, 1H), 3.81-3.62 (M, 4H), 3.5 (M, 1H), 3.1 (M, 1H), 2.4 (SL, 1H), 2.35 (T, J = 7.0 HZ, 2H), 1.75-1.25 (M, 8H); MS 359.9 (M+1).

**Step E:** benzyl (4R)-3-(6-cyanohexyl)-4-formyl-2-oxoimidazolidine-1-carboxylate.

To a solution of benzyl (4R)-3-(6-cyanohexyl)-4-(hydroxymethyl)-2-oxoimidazolidine-1-carboxylate (284.4 mg, 0.79 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (7 ml) was added portionwise Dess-Martin periodinane (336 mg, 0.79 mmole) and the reaction mixture was stirred 1h at rt. Solvent is removed in vacuo, and the residue is triturated with toluene, filtered on celite and solvent removed to give benzyl (4R)-3-(6-cyanohexyl)-4-formyl-2-oxoimidazolidine-1-carboxylate as an oil.

#### EXAMPLE 1

(5S)-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-1-[6-(1H-tetrazol-5-yl)hexyl]imidazolidin-2-one



**Step A:** benzyl (4S)-3-(6-cyanohexyl)-2-oxo-4-[(1E)-3-oxo-4-phenylbut-1-enyl]imidazolidine-1-carboxylate.

To a solution of dimethyl 3-phenyl-2-oxo-propylphosphonate (183 mg, 0.756 mmole) in THF (2 ml) at 0°C was added NaH (60% dispersion in oil, 30.24 mg, 0.793 mmole) and the mixture is stirred at 0°C for 1h. Then a solution of benzyl (4R)-3-(6-cyanohexyl)-4-formyl-2-oxoimidazolidine-1-

carboxylate (270 mg, 0.756 mmole) is added and the mixture is stirred at 0°C for 1h, and 2h at rt. The mixture is then worked-up by adding an ammonium chloride solution (2 ml) and extracted with AcOEt (3x 10 ml), the organic phases are washed with brine, dried on Na<sub>2</sub>SO<sub>4</sub> and the solvent removed. The residue is purified by flash chromatography on silica gel (15 : 85 acetone : toluene) to afford benzyl (4S)-3-(6-cyanoheptyl)-2-oxo-4-[(1E)-3-oxo-4-phenylbut-1-enyl]imidazolidine-1-carboxylate as a oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.45-7.15 (M, 10H), 6.55 (DD, J = 8.4 HZ, 15.5 HZ, 1H), 6.26 (D, 15.5 HZ, 1H), 5.25 (M, 2H), 4.1 (M, 1H), 3.95 (M, 1H), 3.8 (M, 2H), 3.5 (M, 1H), 3.35 (M, 1H), 2.8 (M, 1H), 2.3 (T, J = 7.0 HZ, 2H), 1.6-1.15 (M, 8H) ; MS 474.3 (M+1).

**Step B:** methyl (4S)-3-(6-cyanoheptyl)-4-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidine-1-carboxylate.

To a solution of benzyl (4S)-3-(6-cyanoheptyl)-2-oxo-4-[(1E)-3-oxo-4-phenylbut-1-enyl]imidazolidine-1-carboxylate (132.8 mg, 0.28 mmole) in MeOH (5 ml) at -20°C is added NaBH<sub>4</sub> (17 mg, 0.45 mmole) and the mixture is stirred at -20°C for 1h and allowed to reach rt.. The mixture is then worked-up by adding 0.5 ml acetone and the solvent is removed under reduced pressure. The residue is dissolved in water (2ml) and 1N HCl (1 ml) and extracted with AcOEt (3x 10 ml), the organic phases are washed with brine, dried on Na<sub>2</sub>SO<sub>4</sub> and the solvent removed. The residue is purified by flash chromatography on silica gel (3 : 7 acetone : toluene) to methyl (4S)-3-(6-cyanoheptyl)-4-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidine-1-carboxylate as a oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.35-7.1 (M, 5H), 5.8 (M, 1H), 5.45 (M, 1H), 4.4 (M, 1H), 4.0 (M, 1H), 3.9 (M, 1H), 3.8 (S, 3H), 3.45-3.23 (M, 2H), 2.95-2.7 (M, 3H), 2.3 (T, J = 7.0 HZ, 2H), 2.15 (SL 1H), 1.65-1.2 (M, 8H); MS 400.2 (M+1).

**Step C:** (5S)-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-1-[6-(1H-tetrazol-5-yl)heptyl]imidazolidin-2-one.

To methyl (4S)-3-(6-cyanoheptyl)-4-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-2-oxoimidazolidine-1-carboxylate (55.9 mg, 0.14 mmole) is added Tributylstannylazide (139.5 mg, 0.42 mmole) and the mixture is heated

at 100°C for 3h. The crude mixture is mixed with 3N KOH in MeOH and is stirred at rt for 24h. The solvent is removed under reduced pressure and the residue is dissolved in 1N HCl (7 ml). The aqueous phase is extracted by AcOEt (3x20 ml), and the organic phases are washed with brine, dried on Na<sub>2</sub>SO<sub>4</sub> and solvent removed. Purification by silica gel filtration, gradient (CH<sub>2</sub>Cl<sub>2</sub> : MeOH : 0.1% HCOOH) from (100 : 0) to (94 : 6) to afford (5S)-5-[(1E)-3-hydroxy-4-phenylbut-1-enyl]-1-[6-(1H-tetraazol-5-yl)hexyl]imidazolidin-2-one as an oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.3-7.1 (M, 5H), 5.8 (M, 1H), 5.5 (M, 1H), 5.3 (SL, 1H), 4.45 (M, 1H), 4.05 (M, 1H), 3.45 (M, 1H), 3.2-2.7 (M, 7H), 2.05 (S, 1H), 1.7 (M, 2H), 1.4-1.1 (M, 6H) ; MS 385.4 (M+1).

#### I. Effects of an EP4 Agonist on Intraocular Pressure (IOP) in Rabbits and Monkeys.

##### Animals

Drug-naïve, male Dutch Belted rabbits and female cynomolgus monkeys are used in this study. Animal care and treatment in this investigation are in compliance with guidelines by the National Institute of Health (NIH) and the Association for Research in Vision and Ophthalmology (ARVO) resolution in the use of animals for research. All experimental procedures str approved by the Institutional Animal Care and Use Committee of Merck and Company.

##### Drug Preparation and Administration

Drug concentrations are expressed in terms of the active ingredient (base). The compounds of this invention are dissolved in physiological saline at 0.01, 0.001, 0.0001 % for rabbit study and 0.05, 0.005% for monkey studies. Drug or vehicle aliquots (25 ul) are administered topically unilaterally or bilaterally. In unilateral applications, the contralateral eyes receive an equal volume of saline. Proparacaine (0.5%) is applied to the cornea prior to tonometry to minimize discomfort. Intraocular pressure (IOP) is recorded using a pneumatic tonometer (Alcon Applanation Pneumatograph) or equivalent.



### Analysis

The results are expressed as the changes in IOP from the basal level measured just prior to administration of drug or vehicle and represent the mean, plus or minus standard deviation. Statistical comparisons are made using the Student's t-test for non-paired data between responses of drug-treated and vehicle-treated animals and for paired data between ipsilateral and contralateral eyes at comparable time intervals. The significance of the data is also determined as the difference from the "t-0" value using Dunnett's "t" test. Asterisks represent a significance level of  $p < 0.05$ .

#### A. Intraocular Pressure Measurement in Rabbits

Male Dutch Belted rabbits weighing 2.5-4.0 kg are maintained on a 12- hour light/dark cycle and rabbit chow. All experiments are performed at the same time of day to minimize variability related to diurnal rhythm. IOP is measured before treatment then the compounds of this invention or vehicle are instilled (one drop of 25 ul) into one or both eyes and IOP is measured at 30, 60, 120, 180, 240, 300, and 360 minutes after instillation. In some cases, equal number of animals treated bilaterally with vehicle only are evaluated and compared to drug treated animals as parallel controls.

#### B. Intraocular Pressure Measurements in Monkeys.

Unilateral ocular hypertension of the right eye is induced in female cynomolgus monkeys weighing between 2 and 3 kg by photocoagulation of the trabecular meshwork with an argon laser system (Coherent NOVUS 2000, Palo Alto, USA) using the method of Lee et al. (1985). The prolonged increase in intraocular pressure (IOP) results in changes to the optic nerve head that are similar to those found in glaucoma patients.

For IOP measurements, the monkeys are kept in a sitting position in restraint chairs for the duration of the experiment. Animals are lightly anesthetized by the intramuscular injection of ketamine hydrochloride (3-5 mg/kg) approximately five minutes before each IOP measurement and one drop of 0.5% proparacaine was instilled prior to recording IOP. IOP is measured using a pneumatic tonometer (Alcon Applanation Tonometer) or a Digilab pneumatometer (Bio-Rad Ophthalmic Division, Cambridge, MA, USA).

IOP is measured before treatment and generally at 30, 60, 124, 180, 300, and 360 minutes after treatment. Baseline values are also obtained at these time points generally two or three days prior to treatment. Treatment consists of instilling one drop of 25 ul of the compounds of this invention (0.05 and 0.005 %) or vehicle (saline). At least one-week washout period is employed before testing on the same animal. The normotensive (contralateral to the hypertensive) eye is treated in an exactly similar manner to the hypertensive eye. IOP measurements for both eyes are compared to the corresponding baseline values at the same time point. Results are expressed as mean plus-or-minus standard deviation in mm Hg. The activity range of the compounds of this invention for ocular use is between 0.01 and 100,000 nM.

## II. Radioligand binding assays:

The assays used to test these compounds were performed essentially as described in: Abramovitz M, Adam M, Boie Y, Carriere M, Denis D, Godbout C, Lamontagne S, Rochette C, Sawyer N, Tremblay NM, Belley M, Gallant M, Dufresne C, Gareau Y, Ruel R, Juteau H, Labelle M, Ouimet N, Metters KM. The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim Biophys Acta* 2000 Jan 17;1483(2):285-293 and discussed below:

### *Stable expression of prostanoid receptors in the human embryonic kidney (HEK) 293(EBNA) cell line*

Prostanoid receptor (PG) cDNAs corresponding to full length coding sequences were subcloned into the appropriate sites of the mammalian expression vector pCEP4 (Invitrogen) pCEP4PG plasmid DNA was prepared using the Qiagen plasmid preparation kit (QIAGEN) and transfected into HEK 293(EBNA) cells using LipofectAMINE® (GIBCO-BRL) according to the manufacturers' instructions. HEK 293(EBNA) cells expressing the cDNA together with the hygromycin resistance gene were selected in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % heat inactivated fetal bovine serum, 1 mM sodium pyruvate, 100 U/ml Penicillin-G, 100 µg/ml Streptomycin sulphate, 250 µg/ml active GENETICIN™ (G418) (all from Life Technologies, Inc./BRL) and 200 µg/ml hygromycin (Calbiochem). Individual colonies were isolated after 2-3 weeks of growth under selection using the

cloning ring method and subsequently expanded into clonal cell lines.

Expression of the receptor cDNA was assessed by receptor binding assays.

HEK 293(EBNA) cells were grown in supplemented DMEM complete medium at 37°C in a humidified atmosphere of 6 % CO<sub>2</sub> in air, then  
5 harvested and membranes prepared by differential centrifugation (1000 x g for 10 min, then 160,000 x g for 30 min, all at 4°C) following lysis of the cells by nitrogen cavitation at 800 psi for 30 min on ice in the presence of protease inhibitors (2 mM phenylmethylsulfonylfluoride, 10 µM E-64, 100 µM leupeptin and 0.05 mg/ml pepstatin). The 160,000 x g pellets were  
10 resuspended in 10 mM HEPES/KOH (pH 7.4) containing 1 mM EDTA at approximately 5-10 mg/ml protein by Dounce homogenisation (Dounce A; 10 strokes), frozen in liquid nitrogen and stored at -80°C.

#### Prostanoid receptor binding assays

15 Prostanoid receptor binding assays were performed in a final incubation volume of 0.2 ml in 10 mM MES/KOH (pH 6.0) (EP subtypes, FP and TP) or 10 mM HEPES/KOH (pH 7.4) (DP and IP), containing 1 mM EDTA, 10 mM MgCl<sub>2</sub> (EP subtypes) or 10 mM MnCl<sub>2</sub> (DP, FP, IP and TP) and radioligand [0.5-1.0 nM [<sup>3</sup>H]PGE<sub>2</sub> (181 Ci/mmol) for EP subtypes, 0.7 nM  
20 [<sup>3</sup>H]PGD<sub>2</sub> (115 Ci/mmol) for DP, 0.95 nM [<sup>3</sup>H]PGF<sub>2α</sub> (170 Ci/mmol) for FP, 5 nM [<sup>3</sup>H]iloprost (16 Ci/mmol) for IP and 1.8 nM [<sup>3</sup>H]SQ 29548 (46 Ci/mmol) for TP]. EP<sub>3</sub> assays also contained 100 µM GTPγS. The reaction was initiated by addition of membrane protein (approximately 30 µg for EP<sub>1</sub>, 20 µg for EP<sub>2</sub>, 2 µg for EP<sub>3</sub>, 10 µg for EP<sub>4</sub>, 60 µg for FP, 30 µg for DP, 10 µg for IP and 10  
25 µg for TP) from the 160,000 x g fraction. Ligands were added in dimethylsulfoxide (Me<sub>2</sub>SO) which was kept constant at 1 % (v/v) in all incubations. Non-specific binding was determined in the presence of 1 µM of the corresponding non-radioactive prostanoid. Incubations were conducted for 60 min (EP subtypes, FP and IP) or 30 min (DP and TP) at 30°C (EP subtypes,  
30 DP, FP and TP) or room temperature (IP) and terminated by rapid filtration through a 96-well Unifilter GF/C (Canberra Packard) prewetted in assay incubation buffer without EDTA (at 4°C) and using a Tomtec Mach III 96-well semi-automated cell harvester. The filters were washed with 3-4 ml of the same buffer, dried for 90 min at 55°C and the residual radioactivity bound to  
35 the individual filters determined by scintillation counting with addition of 50 µl

of Ultima Gold F (Canberra Packard) using a 1450 MicroBeta (Wallac).

Specific binding was calculated by subtracting non-specific binding from total binding. Specific binding represented 90-95 % of the total binding and was linear with respect to the concentrations of radioligand and protein used. Total binding represented 5-10 % of the radioligand added to the incubation media.

The activity range of the compounds of this invention for bone use is between 0.01 and 100,000 nM.

## BONE RESORPTION ASSAYS:

### 1. Animal Procedures:

For mRNA localization experiments, 5-week old Sprague-Dawley rats (Charles River) are euthanized by CO<sub>2</sub>, their tibiae and calvariae are excised, cleaned of soft tissues and frozen immediately in liquid nitrogen. For EP<sub>4</sub> regulation experiments, 6-week old rats are given a single injection of either vehicle (7% ethanol in sterile water) or an anabolic dose of PGE<sub>2</sub> (Cayman Chemical, Ann Arbor, MI), 3-6 mg/kg in the same vehicle) intraperitoneally. Animals are euthanized at several time points post-injection and their tibiae and calvariae, as well as samples from lung and kidney tissues are frozen in liquid nitrogen.

### 2. Cell Cultures

RP-1 periosteal cells are spontaneously immortalized from primary cultures of periosteal cells from tibiae of 4-week old Sprague-Dawley rats and are cultured in DMEM (BRL, Gaithersburg, MD) with 10 % fetal bovine serum (JRH Biosciences, Lenexa, KS). These cells do not express osteoblastic phenotypic markers in early culture, but upon confluence, express type I collagen, alkaline phosphatase and osteocalcin and produce mineralized extracellular matrix.

RCT-1 and RCT-3 are clonal cell lines immortalized by SV-40 large T antigen from cells released from fetal rat calvaria by a combination collagenase/hyaluronidase digestion. RCT-1 cells, derived from cells released during the first 10 minutes of digestion (fraction I), are cultured in RPMI 1640 medium (BRL) with 10% fetal bovine serum and 0.4 mg/ml G418 (BRL). These cells differentiate and express osteoblastic features upon retinoic acid treatment. RCT-3 cells, immortalized from osteoblast-enriched fraction III cells, are cultured in F-12 medium (BRL) with 5% Fetal bovine serum and 0.4 mg/ml G418. TRAB-11 cells are also immortalized by SV40 large T antigen from adult rat tibia and are cultured in

RPMI 1640 medium with 10% FBS and 0.4 mg/ml G418. ROS 17/2.8 rat osteosarcoma cells are cultured in F-12 containing 5% FBS. Osteoblast-enriched (fraction III) primary fetal rat calvaria cells are obtained by collagenase/hyaluronidase digestion of calvariae of 19 day-old rat fetuses. See Rodan et al., *Growth stimulation of rat calvaria osteoblastic cells by acidic FGF*, *Endocrinology*, 121, 1919-1923 (1987), which is incorporated by reference herein in its entirety. Cells are released during 30-50 minutes digestion (fraction III) and are cultured in F-12 medium containing 5% FBS.

- 10 P815 (mouse mastocytoma) cells, cultured in Eagles MEM with 10% FBS, and NRK (normal rat kidney fibroblasts) cells, cultured in DMEM with 10% FBS, are used as positive and negative controls for the expression of EP<sub>4</sub>, respectively. See Abramovitz et al., *Human prostanoid receptors: cloning and characterization*. In: Samulesson B. et al. ed) *Advances in prostaglandin, Thrombosznes and leukotriene research*, vol. 23, pp. 499-504 (1995) and de Larco et al., *Epithelioid and fibroblastic rat kidney cell clones: EGF receptors and the effect of mouse sarcoma virus transformation*, *Cell Physiol.*, 94, 335-342 (1978), which are both incorporated by reference herein in their entirety.

### 3. Northern Blot Analysis:

- 20 Total RNA is extracted from the tibial metaphysis or diaphysis and calvaria using a guanidinium isothiocyanate-phenol-chloroform method after pulverizing frozen bone samples by a tissue homogenizer. See P. Chomczynski et al., *Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction.*, *Analyt Biochem*, 162, 156-159 (1987), which is incorporated by reference herein in its entirety. RNA samples (20 mg) are separated on 0.9% agarose/formaldehyde gels and transferred onto nylon membranes (Boehringer Mannheim, Germany). Membranes are prehybridized in Hybrisol I (Oncor, Gaithersburg, MD) and 0.5 mg/ml sonicated salmon sperm DNA (Boehringer) at 42°C for 3 hours and are hybridized at 42°C with rat EP<sub>2</sub> and mouse EP<sub>4</sub> cDNA probes labeled with [<sup>32</sup>P]-dCTP (Amersham, Buckinghamshire, UK) by random priming using the rediprime kit (Amersham). After hybridization, membranes are washed 4 times in 2xSSC + 0.1% SDS at room temperature for a total of 1 hour and once with 0.2xSSC + 0.1% SDS at 55°C for 1 hour and then exposed to Kodak XAR 2 film at -70°C using intensifying screens. After developing the films, bound probes

are removed twice with 0.1% SDS at 80°C and membranes are hybridized with a human GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) cDNA probe (purchased from Clontech, Palo Alto, CA) for loading control.

5     4. In-Situ Hybridization:

Frozen tibiae are sectioned coronally at 7 mm thickness and sections are mounted on charged slides (Probe On Plus, Fisher Scientific, Springfield, NJ) and are kept at -70°C until hybridization. cRNA probes are labeled with <sup>35</sup>S-UTPgS (ICN, Costa Mesa, CA) using a Riboprobe II kit (Promega Madison, WI).

- 10   Hybridization is performed overnight at 50° C. *See M. Weinreb et al., Different pattern of alkaline phosphatase, osteopontin and osteocalcin expression in developing rat bone visualized by in-situ hybridization, J. Bone Miner Res., 5, 831-842 (1990) and D. Shinar et al., Expression of alphav and beta3 integrin subunits in rat osteoclasts in situ, J. Bone Miner. Res., 8, 403-414 (1993), which are both*
- 15   incorporated by reference herein in their entirety. Following hybridization and washing, sections are dipped in Ilford K5 emulsion diluted 2:1 with 6% glycerol in water at 42° C and exposed in darkness at 4° C for 12-14 days. Slides are developed in Kodak D-19 diluted 1:1 with water at 15°, fixed, washed in distilled water and mounted with glycerol-gelatin (Sigma) after hematoxylin staining. Stained sections
- 20   are viewed under the microscope (Olympus, Hamburg, Germany), using either bright-field or dark-field optics.

5. Expression Of EP<sub>4</sub> In Osteoblastic Cell Lines And In Bone Tissue.

- The expression of EP<sub>4</sub> and EP<sub>2</sub> mRNA is examined in various bone
- 25   derived cells including osteoblast-enriched primary rat calvaria cells, immortalized osteoblastic cell lines from fetal rat calvaria or from adult rat tibia and an osteoblastic osteosarcoma cell line. Most of the osteoblastic cells and cell lines show significant amounts of 3.8 kb EP<sub>4</sub> mRNA, except for the rat osteosarcoma cell line ROS 17/2.8. Consistent with this finding, in ROS 17/2.8 cells PGE<sub>2</sub> has no effect on intracellular
- 30   cAMP, which is markedly induced in RCT-3 and TRAB-11 cells. Treatment of RCT-1 cells with retinoic acid, which promotes their differentiation, reduces the levels of EP<sub>4</sub> mRNA. NRK fibroblasts do not express EP<sub>4</sub> mRNA, while P815 mastocytoma cells, used as positive controls, express large amounts of EP<sub>4</sub> mRNA. In contrast to EP<sub>4</sub> mRNA, none of the osteoblastic cells and cell lines express detectable amounts

of EP<sub>2</sub> mRNA in total RNA samples. Expression of EP<sub>4</sub> mRNA in osteoblastic cells, EP<sub>4</sub> is also expressed in total RNA isolated from tibiae and calvariae of 5-week-old rats. In contrast, no EP<sub>2</sub> mRNA is found in RNA from tibial shafts.

5     6. PGE<sub>2</sub> Induces The Expression Of EP<sub>4</sub> mRNA in RP-1 Periosteal Cells And In Adult Rat Tibiae

PGE<sub>2</sub> enhances its own production via upregulation of cyclooxygenase 2 expression in osteoblasts and in bone tissue thus autoamplifying its own effects. PGE<sub>2</sub> also increases the levels of EP<sub>4</sub> mRNA. RP-1 cells are immortalized from a primary culture of adult rat tibia periosteum is examined. These cells express osteoblast phenotypic markers upon confluence and form mineralized bone matrix when implanted in nude mice. Similar to the other osteoblastic cells examined, RP-1 periosteal cells express a 3.8 kb EP<sub>4</sub> transcript. Treatment with PGE<sub>2</sub> (10<sup>-6</sup> M) rapidly increases EP<sub>4</sub> mRNA levels peaking at 2 hours after treatment. PGE<sub>2</sub> has no effect on EP<sub>4</sub> mRNA levels in the more differentiated RCT-3 cells pointing to cell-type specific regulation of EP<sub>4</sub> expression by PGE<sub>2</sub>. EP<sub>2</sub> mRNA is not expressed in RP-1 cells before or after treatment with PGE<sub>2</sub>.

To examine if PGE<sub>2</sub> regulates EP<sub>4</sub> mRNA levels *in vivo* in bone tissue, five-week-old male rats are injected with PGE<sub>2</sub> (3 - 6 mg/Kg). Systemic administration of PGE<sub>2</sub> rapidly increased EP<sub>4</sub> mRNA levels in the tibial diaphysis peaking at 2 h after injection. A similar effect of PGE<sub>2</sub> on EP<sub>4</sub> mRNA is observed in the tibial metaphysis and in calvaria. PGE<sub>2</sub> induces EP<sub>4</sub> mRNA levels *in vitro* in osteogenic periosteal cells and *in vivo* in bone tissue in a cell type-specific and tissue-specific manner. PGE<sub>2</sub> does not induce EP<sub>2</sub> mRNA in RP-1 cells nor in bone tissue.

25     7. Localization of EP<sub>4</sub> mRNA expression in bone tissue

*In situ* hybridization is used in order to localize cells expressing EP<sub>4</sub> in bone. In control experiment (vehicle-injected) rats, low expression of EP<sub>4</sub> is detected in bone marrow cells. Administration of a single anabolic dose of PGE<sub>2</sub> increased the expression of EP<sub>4</sub> in bone marrow cells. The distribution of silver grains over the bone marrow is not uniform and occurs in clumps or patches in many areas of the metaphysis. Within the tibial metaphysis, EP<sub>4</sub> expression is restricted to the secondary spongiosa area and is not seen in the primary spongiosa. Hybridization of similar sections with a sense probe (negative control) does not show any signal.

EP<sub>4</sub> is expressed in osteoblastic cells *in vitro* and in bone marrow cells *in vivo*, and is upregulated by its ligand, PGE<sub>2</sub>.

#### 8. Agonists Of the Present Invention

5           Using standard methods for measuring agonist activity, the following compounds are evaluated in cell cultures and in EP<sub>4</sub> receptor cell-free systems to determine the agonist activity of the compounds in terms of their EC<sub>50</sub> value.